

## ISOMETRIC CONTRACTION OF EPIDERMIS AND STRATUM CORNEUM WITH HEATING\*

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### ABSTRACT

Isometric contraction studies provide valuable data on the response of epidermal tissue to heating in water. A major change occurring in the tissue during heating is a conversion of the molecular structure of the  $\alpha$ -filaments to  $\beta$ -configuration. Prior treatment of the tissue with several solvents show specific effects on the isometric contraction studies and the changes observed are discussed in relation to the structure of the tissue.

The denaturation of proteins by heat in the presence of water involves the loss of secondary and tertiary molecular structure but without chemical degradation (1). The temperature at which denaturation begins (melting temperature) varies with different proteins and is dependent on, among other parameters, the pH and ionic strength of the solution. Some tissues which contain highly oriented fibrous protein, such as tendons, show considerable shortening when heated above the melting temperature as a result of a helix-to-coil transition (2). Rudall (3, 4) reported that epidermis from cow snout epidermis contracted in water with heating and that this was accompanied by a change in structure from  $\alpha$  to cross  $\beta$ . Filaments of  $\alpha$ -protein isolated from snout epidermis also shortened, but at a lower temperature. He also showed epidermis obtained from human back skin behaved similarly, while stratum corneum from palms and soles lengthened. It appeared from Rudall's data that such techniques might be suitable for studying the stability of keratin fibrous proteins in different diseases, and these studies were undertaken to explore that possibility. The present paper indicates the unsuitability of measuring change in length but shows the value of isometric contraction studies in evaluating the effect of temperature on epidermis.

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### MATERIALS AND METHODS

Stratum corneum was obtained from the palms and soles by cutting thin sheets parallel to the surface with a single-edged razor. Since only stratum corneum was removed, the procedure was painless and required no anesthesia. The specimens were stored in sealed vials at 4° C. Tops of blisters resulting from friction or drug eruptions were excised, washed and stored in water at 4° C. The site of blister was designated either as palm and sole or the remainder of the cutaneous surface (body skin). These specimens were used within several days but were found to be stable for weeks at 4° C unless overgrown with microorganisms. Epidermis was obtained from autopsy specimens of body skin by heating at 60° C for 30 secs and gentle scraping with a scalpel.

The specimens were soaked for 48 hours at room temperature in the solvent to be used for the experiment before being tested. Prior treatment with various solvents was carried out for 24 hours.

In the experiments in which change in length was being studied the specimens were clamped at one end and various sized weights attached to the other end. The specimens were immersed in a constant temperature water bath and the changes in length recorded with a cathetometer.

Isometric contraction studies were done with a Statham strain gauge transducer. Specimens (2 mm x 2 cm) were connected by preboiled surgical silk between a glass hook attached to the bottom of a test tube and the micro-scale attachment of the transducer cell. Following adjustment of the tension on the specimen, usually 200 mg, the temperature of the bath was raised 2° C/10 min by advancing the setting of the controller stepwise or continuously with a potentiometer connected to a constant speed motor. Monitoring of the temperature was accomplished with a thermocouple and its output and that of the transducer cell connected to a recorder.

X-ray diffraction studies were performed on dry specimens using nickle-filtered copper K $\alpha$ -

radiation ( $\lambda = 1.54 \text{ \AA}$ ) at 40 KV at a specimen to film distance of 1.50 cm or 5.14 cm.

## RESULTS

### *Effect of Heating on Length*

Specimens of blister-top epidermis from the body skin as well as palms and soles showed no change in length after being immersed in water at 90° C for 1 hour. The load applied to the specimen varied from 50 mg, which produced negligible stretching, to a load sufficient to cause an increase in length of 20% to 30%. Specimens of stratum corneum from palms or soles similarly tested showed a variable increase in length of up to 5%, but no change was observed if the tissue was extracted with chloroform-methanol (3/1) prior to use.

Additional experiments were performed to see if the length of specimens changed following heating in water and subsequent drying. The results are recorded in Table I, and show that no change could be observed in any of the specimens after treatment at 90° C for up to 1 hour.

### *Effect of Heating on Tension*

Epidermis from blisters of body skin was heated in water by raising the temperature setting 2° C every 10 minutes and the change in tension determined (Fig. 1). The initial decrease in tension results from relaxation of the

TABLE I

*The effect of heating on the wet and dry length of epidermis from blisters of body skin*

The specimens were heated at 90° C in water for 1 hour under no tension, and then the final measurements made.

Specimen no.	Initial length (cm)		Final length (cm)	
	Dry	Wet	Dry	Wet
1	2.05	2.20	2.04	2.22
2	2.00	2.18	2.02	2.19
3	2.06	2.22	2.04	2.17
4	2.08	2.23	2.04	2.20
5	2.07	2.24	2.06	2.25

specimen and then there is an increase in tension occurring in a stepwise fashion corresponding to the rise in temperature. The effect of continuous rise in temperature on the tension of blister-top epidermis is shown in Figure 2. Following the initial decrease there is a continuous rise in tension. The initial tension applied to the specimen had no effect on the shape of the curve but only altered the magnitude of the response. The temperature at which the most rapid increase in tension began (inflection point) was estimated as shown in Figure 2, and varied between 82° C to 86° C. Prior extraction of the tissue with chloroform-methanol (3/1) had no effect on the inflection

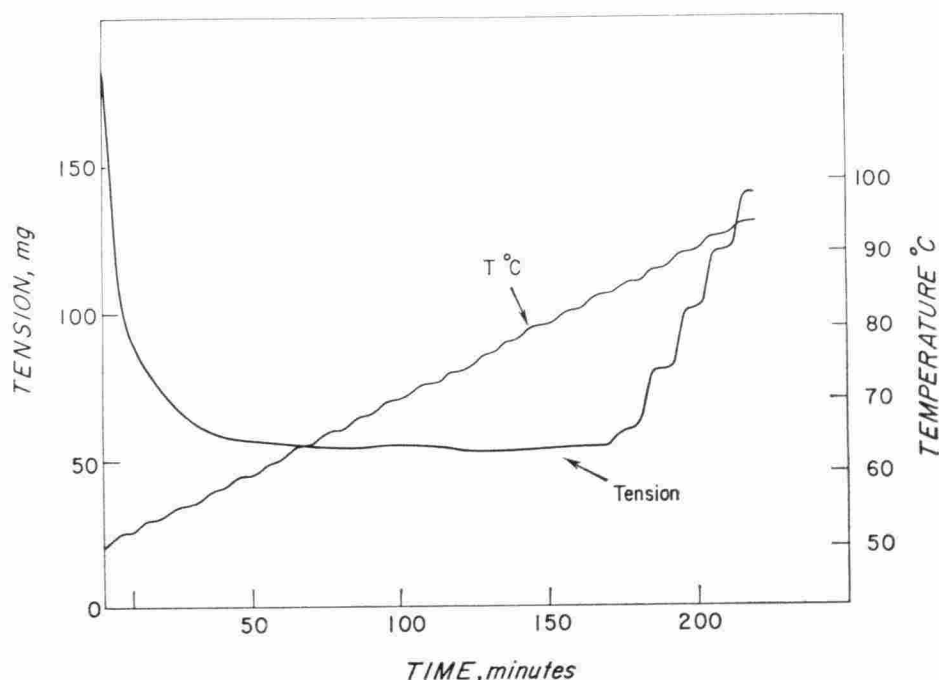


FIG. 1. The effect of stepwise increase in temperature on the tension of blister-top epidermis from body skin.

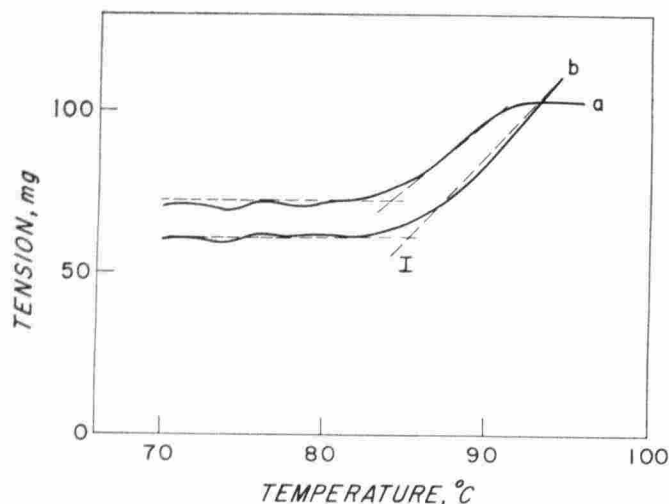


FIG. 2. The effect of a progressive increase in temperature on the tension of blister-top epidermis from body skin. The chloroform-methanol extracted tissue is a and the untreated tissue, b. The inflection point (I) is determined by the intersection of lines drawn through the straightest portions of the curves as indicated.

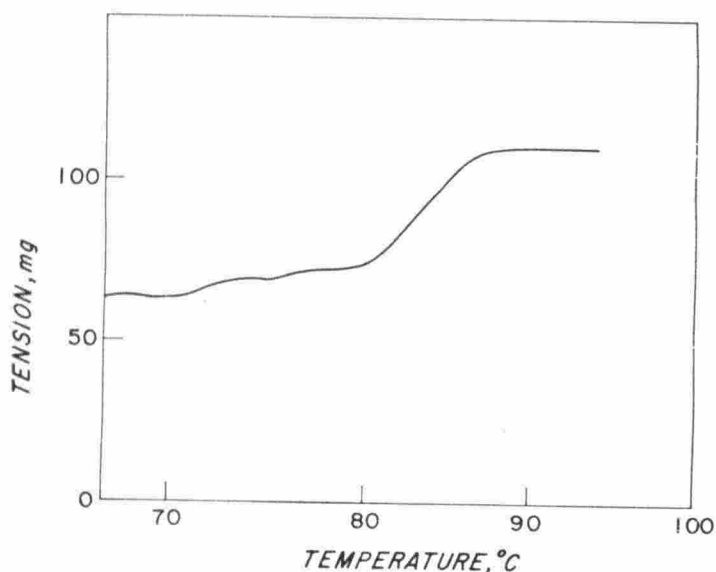


FIG. 3. The effect of a progressive increase in temperature on the tension of chloroform-methanol extracted palmar stratum corneum.

point but resulted in a flattening of the curve at about 90°C (Fig. 2). Experiments with epidermis separated with heat gave similar results.

Stratum corneum obtained from palms and soles showed a continuous decrease to essentially zero tension with heating, which was associated with swelling and elongation of the specimens. Prior treatment of the tissue with hexane had no effect on the results but chloroform-methanol (3/1) extraction resulted in isometric contraction as seen in Figure 3. The curve obtained after extraction showed an inflection point at 76°C to 80°C and then flattening below 90°C. In addition, the curves from many specimens showed a region with a slower in-

crease in tension starting at about 72°C. These results were obtained with stratum corneum from individuals with varying thickness of the epidermis and of different age and sex.

Epidermis from blisters of the palms and soles gave results similar to those obtained from blisters of the body skin with an inflection point between 82°C and 85°C, and with progressive increase in tension at higher temperatures. There was no variation in different areas of the palms and soles. Treatment with chloroform-methanol (3/1) also resulted in a flattening of the curve at higher temperatures.

The results obtained with the various tissues are summarized in Table II so that the significant features may be more clearly discerned.

#### Effect of pH

The effect of pH on isometric contraction was studied by heating epidermis from blisters of the palm in a variety of buffers. Within the pH range 4 to 8 (.1 M citrate pH 4 to 6, .1 M phosphate pH 6 to 7, and .1 M Tris, pH 7 to 8.5), the results were the same as in water as described above. The curves for pH 3.0 and 3.5 (.1 M citric acid-sodium citrate) showed no increase in tension until 90°C and the magnitude of the change above this was quite small. At pH 8.5 (.1 M Tris) and greater, there was a decrease in tension in the temperature range in which contraction was normally observed and a small rise at much higher temperature. Specimens soaked in buffer below pH 3 or above pH 8 and then washed exhaustively in distilled water showed a normal response when heated in distilled water.

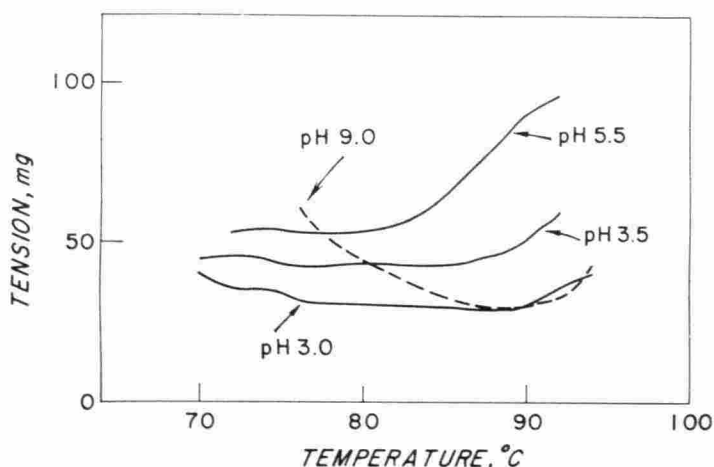


FIG. 4. The effect of pH on the isometric contraction curve.

TABLE II  
Summary of isometric contraction studies

Tissue	Heating in water 2° C/10 min.	Extraction in chloroform-methanol (3/1) and then heating in H <sub>2</sub> O, 2° C/10 min.
Stratum corneum, palms or soles	Continuous decrease in tension	Increase in tension starting at 76–80° C and complete by 90° C
Blister-top, palms or soles	Continuous increase in tension, starting at 82°–85° C	Temperature of initial increase in tension unaffected but contraction completed by 90° C
Blister-top, body skin	Continuous increase in tension, starting at 82°–86° C	Temperature of initial increase in tension unaffected but contraction completed by 90° C
Heat-separated epidermis	Continuous increase in tension, starting at 82°–86° C	Temperature of initial increase in tension unaffected but contraction completed by 90° C

### Effect of Ionic Strength

The effect of ionic strength on the contraction of palmar stratum corneum was studied by equilibrating specimens of this material in solutions of varying concentration of sodium chloride for 48 hours at room temperature and then heating the tissue in that solution. At low salt concentration no increase in tension with heat was observed but from 2 M to 4 M sodium chloride the tissue showed isometric contraction starting at 78° C  $\pm$  2° C. The results observed with blister-top and heat-separated epidermis were quite different from this. At low salt concentrations the results were similar to experiments done in water, while in 4 M sodium chloride the temperature of the inflection point was reduced to 70° C. The effect of salt was reversible if the specimens were thoroughly washed with water.

### Effect of Treatment with Urea

Specimens of epidermis from blisters of the palm were soaked in 6 M urea for 48 hours at room temperature and then soaked in distilled water for 24 hours. Control specimens soaked in water for 3 days showed an inflection point at 82° C to 85° C while treated specimens showed the change in tension at 70° C to 72° C.

### X-Ray Diffraction Analysis

Epidermis from blister tops and samples of stratum corneum from palms or soles were studied by x-ray diffraction analysis following completion of the isometric contraction studies. All specimens heated above 85° C showed loss

or diminution of the 5.15 Å  $\alpha$ -reflection and the development of a new sharp reflection at 4.65 Å accentuated on this meridian. In the case of stratum corneum from palms and soles, this change in the x-ray diffraction pattern was present whether or not increase in the tension of the specimen was observed. Similarly, specimens studied below pH 4 and above pH 9 which did not demonstrate an increase in tension also revealed a change in x-ray diffraction pattern. The lowest temperature at which there was a change to the cross  $\beta$  structure was difficult to determine because of the inherent problem of appreciating small differences in the x-ray pattern with photographic techniques. However, it appears that no change can be observed below the temperature of the inflection point. X-ray changes were also observed in specimens which were heated without being under tension.

### DISCUSSION

The response of epidermis to heat is a complex process which must involve several changes in the tissue. The loss of the  $\alpha$ -helix and the appearance of the cross  $\beta$  structure is a constant feature of the response to heating and explains the large increase in tension which is often observed. No measureable shortening of the specimens could be observed, however, which is in contrast to the earlier observations of Rudall (3, 4). No explanation of this disparity is apparent, except Rudall has stated that recent attempts to repeat his previous work have met with technical difficulties (5). The failure of epidermis to shorten despite



folding of the fibrous protein may result from the type of orientation observed in the stratum corneum. The filaments lie parallel to the surface but have no preferred orientation in that plane (6). Although stretching does induce some directional orientation, the degree of filament alignment is not great and probably insufficient to produce an overall shortening of the specimen.

The response of palmar stratum corneum appears to be related to a large increase in water uptake by the tissue during heating. Treatment of the stratum corneum with chloroform-methanol, which is known to result in loss of components with a high water binding capacity (7), eliminates the swelling and isometric contraction can then be observed. The difference in the response of epidermis from blisters of palm and sole and slices of stratum corneum cannot be explained by differences in thickness. Scraping the bottom of the blister roof or gentle digestion with 0.1% trypsin to remove the viable epidermal cells has no effect. Drying the specimen and then rewetting also does not alter the results. These data suggest that the whole stratum corneum is not uniform and that the lowermost layers behave differently than the upper ones. Since it is unlikely that upper layers of the stratum corneum acquire new constituents which imbibe water, it seems reasonable that in lower layers uptake of water is limited, perhaps by diffusion, so that swelling does not occur. The contraction in this zone from the melting of the  $\alpha$ -helix is apparently able to overcome the swelling effect of the upper areas.

It has not been possible to obtain sheets of superficial stratum corneum from normal body skin, but heat separated and blister-top epidermis behave quite similar to blisters of the palm or sole.

Treatment of blister-top epidermis with chloroform-methanol (3/1) appears to have no effect on the inflection point. This would suggest that the lower inflection point observed for delipidized palmar stratum corneum does not result from loss of some lipid components but is a property of the protein components. A possible explanation is that the tissue is being continuously denatured while it remains on the surface of the skin and this results in loss of stability. There is one feature of the isometric contraction curve which is clearly

altered following treatment of the tissue with chloroform-methanol mixture, the continuous increase in tension above 90° C. These results suggest that this may represent a phase change in some lipid component of tissue.

Below pH 4 and above pH 8.5, there was a profound effect on the initial contraction of the tissue. The lack of contraction cannot be explained as a direct effect of pH on altering the  $\alpha$ -structure since x-ray diffraction analysis of treated but unheated tissue showed no change in molecular structure. This pH effect has several possible explanations, among which are: 1) differences in binding of water; 2) alteration of packing of filaments; and 3) slippage of the filaments. The present data do not permit a distinction between these and other possibilities.

The contraction of palmar stratum corneum during heating in concentrated salt solutions most likely results from the high osmotic pressure of the environment and the limited availability of free water to the tissue. In experiments with blister-top epidermis an additional effect of high salt concentrations could be observed. There was a decrease in the temperature of the inflection point which may have resulted from neutralization of the charge of the filaments and loss of stability.

Extracting the tissue with 6 M urea and subsequent washing with water produces a decrease in the temperature of the inflection point. Tissue treated in this way still shows an  $\alpha$ -pattern by x-ray diffraction and there appears to be no permanent damage to the helical structure. However, several protein components of the epidermis are removed by this procedure (8). Loss of one or more of these components may produce a decreased stability of the helix and result in a lower inflection point temperature. This suggests that the character of the isometric contraction curve is not only related to the inherent properties of the filaments but also their interaction with other cellular proteins.

The x-ray changes observed in epidermis with heating are quite different than those noted in hair and nails. In the latter two tissues no loss in the  $\alpha$ -structure is observed below 100° C. X-ray changes are observed in hair above 130° C and the x-ray diffraction pattern shows a parallel B configuration with accentuation of the 4.65 Å reflection on the equator (9). Epidermis and stratum corneum heated above the inflection point show loss of the 5.15 Å reflec-

tion and appearance of a sharp reflection at 4.65 Å. However, when the heated tissue is stretched in water and dried, the x-ray diffraction pattern shows accentuation of the 4.65 Å reflection on the meridian. This is the pattern of the cross  $\beta$  protein in which the polypeptide chains fold back on themselves. The cross  $\beta$  configuration can be induced in the  $\alpha$ -protein of hair by a variety of techniques, but is not seen following simple heating. Epidermis has significantly fewer disulfide cross links than hair (10, 11) and this may be the explanation for the formation of a cross  $\beta$  rather than the parallel  $\beta$  configuration.

The techniques described in this paper may be of value in studying the epidermis and stratum corneum of certain disorders of keratinization. The uptake of water, the temperature of isometric contraction, the response at temperatures above 90° C, and the effect of treatment of the tissue with a variety of solvents may provide further information on the defects at a molecular level in keratinized tissue.

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